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## ABSTRACT

A method for the stepwise creation of phosphodiester bonds between desired nucleosides resulting 5 synthesis of polynucleotides having a predetermined nucleotide sequence by preparing an initiation substrate containing a free and unmodified 3'-hydroxyl group; attaching a mononucleotide selected according to the order of the predetermined nucleotide sequence to the 10 hydroxyl of the initiating substrate in a solution containing a catalytic amount of an enzyme capable of catalyzing the 5' to 3' phosphodiester linkage of the 5'phosphate of the mononucleotide to the 3'-hydroxyl of the initiating substrate, wherein the mononucleotide contains 15 a protected 3'-hydroxyl group, whereby the protected mononucleotide is covalently linked to the initiating substrate and further additions are hindered by the 3'hydroxyl protecting group. Methods in which mononucleotide immobilized on a solid support is added to 20 a free polynucleotide chain are also disclosed.